[processing] comparing the first and second portions of the modulated

B9 **[return radiation]** fluorescence, using a predictive model, to determine a physiological characteristic of the sample;

wherein the predictive model is multivariate.

Please add new claim 61, as follows:

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~~61~~. (New) A spectroscopic method of analyzing a sample, comprising:

irradiating a sample with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by the sample;

monitoring a first portion of the modulated fluorescence at a first angle

B10 from the sample

monitoring a second portion of the modulated fluorescence at a second angle from the sample

comparing the first and second portions of the modulated fluorescence to determine a modulation characteristic of the sample.

REMARKS

In view of the foregoing amendment which canceled claims 4 and 11 and added new claim 61, the claims pending in the instant application are: 1-3, 5-10, 12-42, 48-61.

Pursuant to the Office Action of August 16, 1999, claims 8-10, 35, 40, 42, 49, 51, 59 and 60 are allowed. Furthermore, claims 25-28 are objected to as being dependent upon a rejected base claim. However, in light of the allowance of claim 8 which serves as the base claim of claims 25-28, Applicants respectfully submit that these claims should also be allowed.

As for the remaining pending claims of the instant application which were rejected under Sections 102(a), 102(b), 102(e) and/or 103(a), Applicants respectfully submit that these claims as amended hereinabove and supported by the observations set forth below define patentable subject matter. Applicants also herewith submit a Declaration by Ramez E. Shehada, Ph.D., a named inventor of the instant application, setting forth detailed observations on the cited references Zuckerman, Alfano et al. and Sevick-Muraca et al.

A. Claims Defining Patentable Subject Matter Over Zuckerman Cited Under Section 102(b):

Of the claims rejected under Section 102(b) as being anticipated by Zuckerman, Applicants focus on independent claims 1, 39, 41, 48, 50 and 52. In particular, each of these base claims provides for the monitoring of modulated fluorescence from a first and a second distances from the sample, and the comparing of the modulated fluorescence monitored at the first and second distances. In that regard, Applicants observe that Zuckerman makes no mention whatsoever of monitoring a modulated fluorescence from a first and second distances from the sample, much less of comparing such modulated fluorescence. Therefore the instant invention is distinct from Zuckerman on at least these two features.

On the first feature of monitoring fluorescence from a first and a second distances from the sample, Applicants reemphasize that Zuckerman's Figure 3 and the corresponding text at column 10, lines 1-48, as referenced by the Examiner, describe the returned fluorescence not as being measured from the sample at all, but rather as measured from a single location within the tip 68 of the catheter. In fact, the text of Zuckerman cited by the Examiner indicates that:

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(1) the fluorescence is emitted from the probe tip 68 ("fluorescence emission from the probe tip 68"), and not from the biological sample (See, par. 8, Decl. Of Shehada);

(2) the emitted fluorescence is collected from the probe tip 68 by a single optical fiber ("fluorescence emission from the probe tip 68 returns along the single mode polarization-preserving glass fiber"), and hence it is not collected at first and second distances from the sample (See, par. 7, Decl. Of Shehada); and

(3) the optical detectors 88A and 88B detects the linearly polarized vector components $I_{||}$ (parallel 86A) and $I_{\perp}$ (perpendicular 86B) of the returned fluorescence ("a Wollaston prism polarizer 84, which resolves the emitted fluorescence into its linearly polarized components parallel 86A and perpendicular 86B to the plane of excitation polarization"). Hence the detectors 88A and 88B do not detect the fluorescence of the irradiated sample from a first and second distances from the sample (See, par. 5, Decl. Of Shehada).

On the second feature of comparing the modulated fluorescence, little, if anything, can be said on Zuckerman for it is completely devoid of any relevant disclosure or teaching of a comparison between the measured fluorescence.

Accordingly, as each of independent claims 1, 39, 41, 48, 50 and 52 provides for the step of monitoring modulated fluorescence at a first and a second distances from the sample and comparing such modulated fluorescence, each defines not only novel subject matter, but nonobvious subject matter neither contemplated nor suggested by Zuckerman. With independent claim 1 serving as base claim for claims 2-7, 11-24, 29-34, 36-38 and 57 and independent claim 52 serving as base claim for claim 58, it is respectfully

submitted that these dependent claims define patentable subject matter, as well.

B. Claims Define Patentable Subject Matter over Alfano et al. Cited Under Section 102(e):

Without any admission on the alleged priority of Alfano et al. under Section 102(e) Applicants hereinbelow distinguish the subject matter defined by independent claims 1, 53, 54, 55 and 56. Again, each of these base claims provides for the monitoring of modulated fluorescence from a first and a second distances from the sample, and the comparing of the modulated fluorescence monitored at the first and second distances. In contrast, Alfano et al. make no disclosure or suggestion of monitoring the modulated fluorescence at a first and second distances from the irradiated sample, or of comparing such modulated fluorescence. In fact, according to the embodiment shown in Figure 2 and described in column 5, the returned fluorescence is measured from the same irradiated location on the excised (dead) tissue specimen on a slide. Figure 2 of Alfano et al. is described with "the light transmitted from light source 43 is sent through a first leg 45 of a trifurcated fiber optic bundle 47. Disposed within first leg 45 is a filter 49, which is selective for light of a wavelength which will cause the dye in the sample to fluoresce, i.e. at a wavelength within the absorption curve for the dye" (column 5, lines 39-44). In particular, "The light emergent from the probe end 48 of bundle 47 illuminates a small portion of the slide S upon which the sample is smeared." (column 5, lines 53-55). (See, par. 12, Decl. Of Shehada).

Throughout the text of columns 4 and 5 of Alfano et al., disclosure is made of use of the same "probe end" 48 for illumination and collection of the fluorescence emitted from the illuminated small portion of the slide S. Whereas the fluorescence collected by the

single "probe end" 48 is split into two portions that are transmitted to the second leg 51 and a third leg 53 of bundle 47, the purpose of splitting the fluorescence into two portions is to enable its filtering by two different filters 55 and 57 and hence isolate the wavelengths that are characteristic to the dye and the native fluorescence of the cells in sample S, respectively (see column 5, lines 58-63). (See, par. 10, Decl. Of Shehada).

Moreover, the disclosure in Alfano et al. of "The light passing through filters 55 and 57 is detected by photomultiplier tubes 59 and 61, respectively, and converted into electrical signals, which are transmitted to electronics lock-in and then processed by computer 52" (column 5, lines 64-67) indicates that the photomultiplier tubes 59 and 61 detect the fluorescence intensity passing through filters 55 and 57, respectively. It should be noted that both fluorescence portions measured by the photomultipliers 59 and 61 had originated from the same location (i.e. the fluorescing area facing the "probe end" 48 on the sample S. (See, par. 9, Decl. Of Shehada). However, as indicated above, each portion has been selectively filtered to contain only the wavelengths λ_D and λ_N that are characteristic to the dye and the native fluorescence of the cells, respectively.

Furthermore, the system in FIG. 3 of Alfano et al., at a given time, can only scan (i.e. illuminate and probe) a small portion of the slide S (i.e. that portion facing the "probe end" 48). In order to scan a larger area of the slide S, the slide is moved in X-Y directions underneath the "probe end" 48 and a scan is performed at each x-y location to determine the type of cell (i.e. benign or malignant) in front of the "probe end" 48. Note that each scan of a given location is processed separately and independently from preceding and subsequent scans performed on neighboring locations. (See, par. 11, Decl. Of Shehada).

In view of the foregoing, it can be seen that Alfano et al. did not use, imply or anticipate monitoring modulated fluorescence at first and a second distance from the

irradiated sample and comparing such modulated fluorescence, the combination of which are provided for in Applicants' base claims 1, 53, 54, 55 and 56. Accordingly, it is respectfully submitted that claims1-7, 11-22, 30, 31, 36-38, and 53-56 are not anticipated nor rendered obvious by Alfano et al.

C. Claims Define Patentable Subject Matter Over Sevick-Muraca et al.

Without any admission on the alleged priority of Sevick-Muraca et al. under Section 102(a), Applicants distinguish base claims 1, 52, 53, 54 and 56. Again, each of these base claims provides for the monitoring of modulated fluorescence from a first and a second distances from the sample, and the comparing of the modulated fluorescence monitored at the first and second distances. In contrast, Sevick-Muraca et al. fail to disclose or teach the comparison of the returned radiation collected from two detection sites. Throughout pages 7, 8 and 9, Sevick-Muraca suggest the use of multiple detection sites solely for the purpose of imaging the whole sample. See, for example:

Page 7, lines 14 -16 ("System 110 includes modulated light source 120 to supply an intensity modulated excitation light of predetermined frequency and wavelength to tissue 100 via fiberoptic 123.");

Page 7, lines 19 -20 ("Beam splitter 126 may be employed to direct a small portion of the excitation signal to reference sensor 128 for processing purposes.");

Page 7, lines 20-22 ("System 110 also includes detection subsystem 140 which has optic fibers 143 to detect photons emitted from tissue 100 from a number of corresponding detection sites.");

Page 8, lines 1-4 ("Sensors 128, 148 and source 120 are operatively coupled to heterodyne subsystem 130. Subsystem 130 is configured to obtain information about the phase, AC, and DC intensity of light detected with sensor 128 relative to light

detected with the sensor 148 using conventional laser heterodyning techniques."); and

Page 9, lines 8-10 ("In stage 216, the phase θ_{obs} and log of AC intensity M_{obs} of the emission at each detection site "i" relative to the excitation light source 120 are determined at the heterodyne (or offset) frequency.").

The foregoing text from Sevick-Muraca et al. demonstrates the disclosure and teachings thereof of using intensity modulated excitation light to excite the sample 100 to emit fluorescence, where the excitation light is transmitted to the sample via the fiberoptic 123 and simultaneously detected by sensor 128 to be used as reference. The generated fluorescence is collected by optical fiber(s) 143 detected with the sensor(s) 148. The heterodyne subsystem 130 processes the fluorescence emission from each detection site "i" relative to the excitation light detected by sensor 128 to determine the (a) phase θ_{obs} and (b) log of AC intensity M_{obs} of the emission from that site "i". The θ_{obs} and M_{obs} measured from a given detection site "i" are used in an iterative calculation process (successive iterations loop 220 in Figure 2) to estimate the (1) quantum yield* and the (2) lifetime of the fluorescence at that detection site. Hence, it cannot be overemphasized that the θ_{obs} and M_{obs} measured from a given detection site are processed <u>separately and independently</u> from those measured at the other detection sites. The <u>only purpose</u> for Sevick-Muraca et al. to use <u>multiple detection sites is to determine the spatial variation</u> (or image) of the (1) <u>quantum yield</u> and the (2) <u>lifetime</u> of the fluorescence throughout the sample. (See, par. 14, Decl. Of Shehada)

Advantageously, Applicants' claimed invention monitors the fluorescence intensity at two different detection sites about the fluorescence volume <u>and compares them</u> to determine a modulation characteristic of the sample. As shown by the above discussion and cited text, Sevick-Muraca et al. did not use, imply or anticipate the

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comparison of the fluorescence emitted from two detection sites to determine the fluorescence characteristic of the sample. Accordingly, Applicants respectfully submit that base claims 1, 52, 53, 54, 55 and 56 are not anticipated nor rendered obvious by Sevick-Muraca et al.

D. Observations of Powers cited under Section 103(a):

Claims 29-34 are rejected under U.S.C. 35 U.S.C. § 103(a) as being unpatentable over Zuckerman in view of Powers. Since claims 29-34 incorporate all the elements of claim 1, the observations of Zuckerman set forth above are hereby incorporated. As for Powers, one of ordinary skill in the art would not have combined Zuckerman and Powers to arrive at the subject matter of claims 29-34 because of the following reasons.

Firstly, Powers discloses an apparatus to excite a non-living sample with electromagnetic radiation <400nm and measure the induced fluorescence of NADH generated by microbes on a non-living surface. As such, it would not have been possible by one of ordinary skill in the art to modify the method of Zuckerman to measure the intrinsic fluorescence in light of the disclosure of Powers. This is simply because Zuckerman determines the oxygen concentration of a sample by measuring changes in the anisotropy of an O_2-quenchable probe substance (i.e. pyrenebuteric acid or pyrene) that is a part of the measuring apparatus (i.e. exogenous to the sample). On the other hand, Powers disclose a method to measure the concentration of NADH that is indigenous to the sample being tested.

Moreover, Powers did not disclose a method and apparatus for determining the intrinsic fluorescence of the sample as indicated by the Examiner. Powers makes no mention whatsoever of the word "intrinsic"

throughout the whole patent. In that regard, intrinsic fluorescence of the instant invention relates to the substantially pure fluorescence generated by the fluorophores of a sample. Immediately after its generation, the intrinsic fluorescence suffers wavelength-dependent attenuation by local chromophores and scatterers. Hence, the intrinsic fluorescence cannot be readily measured, however, it can be obtained from its measurable attenuated version if the local attenuation can be determined, which was made possible by the instant invention. In that regard, Powers does not disclose any methods to measure the intrinsic fluorescence, but rather it suggests normalizing the returned fluorescence signals by the reflected excitation signal to compensate for: (1) variations in the distance of the probe **167** from the surface and (2) variations between different surfaces as quoted below (this has little relevant to the intrinsic fluorescence of the instant invention). See column 4, lines 43-46 of Powers.

Accordingly, the subject matter of claims 29-34 would not have been rendered obvious by Zuckerman in view of Powers, as the claims provide for the steps of monitoring the modulated fluorescence at a first and a second distances from the irradiated sample, and comparing such modulated fluorescence for determining a modulation characteristic or intrinsic fluorescence.

E. New Claim 61 Defines Patentable Subject Matter

Applicants have introduced new claim 61 which provides for the step of monitoring a first portion of the modulated fluorescence at a first angle from the sample, monitoring a second portion of the modulated fluorescence at a second angle from the sample and comparing the first and second portions of the modulated fluorescence to determine a modulation characteristic of the sample. Applicants submit that this method is also novel and nonobvious in view of the

cited references.

F. Summary

In view of the foregoing, Applicant requests reconsideration and allowance of claims 1-7, 11-24, 29-34, 36-39, 41, 48, 50, 52-61, in addition to allowed claims 8-10, 25-28, 35, 40, 42, 49 and 51. The Examiner is invited to telephone the undersigned at (213) 622-7700, extension 114, to resolve any outstanding issues.

Respectfully submitted,

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